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APPENDIX 1: LIST OF PAPERS PRESENTED

1. Poster presentation at 2nd International Symposium on Medicinal and Nutraceutical Plants (2nd ISMNP)
Title: Antioxidant Activity of *Beta vulgaris* (Beetroot)
Venue: All Indian Institute of Medical Sciences (AIIMS), New Delhi, India
Date: 25-27 November 2009

2. Poster presentation at International Conference on Natural Products (ICNP)
Title: Comprehensive Study on the Antioxidant Property of *Beta vulgaris* (Beetroot)
Venue: Bayview Beach Resort, Batu Ferringhi, Penang
Date: 11-12 December 2010

3. Oral presentation at 15th Biological Sciences Graduate Congress (BSGC)
Title: Detailed Study on the Antioxidant Property of *Beta vulgaris* (Beetroot)
Venue: Institute of Biological Sciences, University of Malaya, Kuala Lumpur
Date: 15-17 December 2010

4. Poster presentation at One day scientific and motivation seminar by Cenar (Centre for natural products and drug discovery, UM)
Title: Comprehensive Study on the Antioxidant Property of *Beta vulgaris* (Beetroot)
Venue: Crystal Crown Hotel, Petaling Jaya, Kuala Lumpur
Date: 22nd December 2010

5. Poster presentation at 25th Scientific Meeting of Malaysian Society of Pharmacology and Physiology (25th MSPP)
Title: Evaluation of Lipid Oxidation Inhibition Activity of *Beta vulgaris L.* (beetroot)
Venue: University Putra Malaysia (UPM)
Date: 25-26 May 2011

6. Poster presentation at 16th Biological Sciences Graduate Congress (16th BSGC)
Title: Evaluation of Lipid Oxidation Inhibition Activity of *Beta vulgaris L.* (beetroot).
Venue: National University of Singapore (NUS)
Date: 12-14th Dec 2011

APPENDIX 2: Preparation of Reagents

1. Antioxidant Assays

DPPH (1,2-dipheyl-2-picrylhydrzyl) Radical Scavenging Assay

Ascorbic Acid / Butylated Hydroxyanisole (BHA)

A stock of ascorbic acid/BHA (Sigma) in methanol was prepared at a concentration of 400 µg/ml by diluting 0.04 g ascorbic acid/BHA in 100 ml of methanol. The stock solution was kept in Duran bottle wrapped with aluminium foil. This is to ensure that there is no degradation activity as it is easily degraded in the presence of light (light sensitive compound). The preparation of the stock is also conducted in a dark place.

1,2-Diphenyl-2-picrylhydrzyl (DPPH)

A stock of DPPH (Sigma) in methanol was prepared at concentration of 8mg/ml (0.08g + 10 ml of methanol). The stock solution was kept in Duran bottle wrapped with aluminium foil. This is to ensure that there is no degradation activity as it is easily degraded in the presence of light (light sensitive). The preparation of stock is also conducted in a dark room.

2. Reducing Power Assay

Potassium Ferricyanide (1%,w/v) $K_3Fe(CN)_6$

Potassium Ferricyanide 1% was prepared by dissolving 0.1 gm of Potassium Ferricyanide (Sigma) in 10ml distilled water. The solution was kept in centrifuge tube and wrapped with aluminium foil.

Trichloroacetic acid (10%, w/v)

TCA 10% was prepared by dissolving 5gm of TCA (Sigma) in 500ml distilled water. The solution was kept in flask wrapped with aluminium foil.

Ferric Chloride (0.1%,w/v) $FeCl_3.6H_20$

Ferric chloride solution was prepared by dissolving 0.01gm of ferric chloride (Sigma) in 10ml distilled water. The solution was kept in centrifuge tube and wrapped in aluminium foil.

0.2M Phosphate Buffer pH6.6

i. 0.2M Monobasic Stock

0.2M Monobasic Stock was prepared by dissolving 27.80gm of sodium phosphate monobasic (NaH_2PO_4, Sigma) in 1000ml distilled water.

ii. 0.2M Dibasic Stock

0.2M Dibasic Stock was prepared by dissolving 53.65gm of sodium phosphate monobasic (Na_2HPO_4, Sigma) in 1000ml distilled water.

iii. 0.2M Phosphate Buffer pH6.6

0.2M Phosphate Buffer pH6.6 was prepared by adding 62.50ml 0.2M monobasic stock and 37.50ml 0.2M dibasic stock in 200ml distilled water. The pH of the solution was calibrated to pH6.6 by using a pH meter in the laboratory.

3. β-carotene bleaching assay

0.2mg/ml β-carotene solution
0.2 mg/ml of β-carotene solution was prepared by dissolving 0.002 mg of β-carotene in 10 ml chloroform. The solution was kept in a centrifuge tube and wrapped in aluminium foil to avoid any unwanted degradation to the solution by light.

Tween 80
Tween 80 (polyoxyethylene sorbitan monolaurate)

Linoleic acid
Linoleic acid is obtained from Sigma. Best kept in ice throughout experiment and kept in -20˚C when not in use.

4. Metal Chelating Assay

Preparation of EDTA (Ethylenediaminetetraacetic acid)
1 g of EDTA is dissolved in 4 ml distilled water. The pH is adjusted to pH 8 by using natrium hydroxide (NaOH) while stirring. EDTA will start dissolve completely once the pH has reach pH 8. Once the EDTA dissolved, the total volume is made up to 10 ml with distilled water.

Preparation of Ferrozine (3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p′*-disulfonic acid monosodium salt hydrate) (5 mM)
0.0024625 g of ferrozine was diluted with deionized distilled water.

Preparation of ferric chloride ($FeCl_2$) (2 mM)
0.039762 g of ferric chloride crystal which is light green in colour is diluted with 100 ml of deionized distilled water. This mixture must be left standing for some time for the crystals to be completely diluted.

Preparation of deionized water
Deionized water (18 megaohm-cm) was prepared using machine. Dionized water is used to avoid contaminations by ions outside of the reaction system that might affect the results of the experiments.

5. **Superoxide dismutase (SOD) activity assay**

Preparation of working solutions

WST working solution
This is prepared by diluting 1 ml of WST solution with 19 ml of Buffer Solution. This solution has the ability to be stable for almost 2 months at the temperature 4ºC.

Enzyme Working Solution
The Enzyme Solution tube was initially centrifuged for 5 seconds. The Enzyme Solution (15 µl) was then pipetted and mixed with 2.5 ml of dilution buffer. This solution was stable for 3 weeks at 4ºC.

6. **Total Phenolic Content (Folin-Ciocalteau Assay)**

Preparation of Gallic Acid Solution
0.5 g of dry gallic acid, which is also known as 3,4,5-trihydroxybenzoic acid is diluted with 10ml of absolute ethanol. The mixture is then made into 100ml with distilled water. This preparation can be kept in refrigerator for 2 weeks.

Preparation of Saturated Sodium Carbonate Solution (~35%)
200 g of anyhydrous sodium carbonate is added into 800 ml of water. It is then brought into boiling and then left to cool thereafter. After the solution is cooled, a few crystals of sodium carbonate were added and it is left standing at room temperature for 24 hours. After 24 hours, the thick sodium carbonate solution is filtered and water is added to make the whole solution to 1 liter.

Folin-Ciocalteau Phenol Reagent (Sigma)
It contains phoshomolybdate and phosphotungstate. Commonly used for colorimetric assay to determine phenolic and polyphenolic antioxidants.

7. **Thiobarbituric Acid Reactive Species (TBARS) Assay**

Egg yolk suspension
A fresh egg was purchased and rinsed. The egg was broken and egg white is discarded. Only the egg yolk was taken and used for the experiment. The egg yolk was weighed and the weight of the egg yolk noted. Dilution with PBS was conducted at the ratio 1:1. The diluted egg yolk was then mixed well using a magnetic stirrer. This egg yolk was then diluted again 40x using PBS (pH 7.4) to obtain yolk suspension.
Method of egg yolk suspension preparation
Let say the weight of egg yolk is 13.4 g
The egg yolk would be diluted with PBS (pH 7.4) at 1:1 ratio; 13.4 g was added with 13.4 ml of PBS. This is then stirred using a magnetic stirrer.
To obtain egg suspension, the diluted egg yolk was then diluted again 40x (1 ml of egg yolk added into 39 ml of PBS) using PBS. In this experiment, 3 ml of the diluted egg yolk is added to 117 ml of PBS.

0.8% 2-Thiobarbituric acid
2-Thiobarbituric acid (0.8 g) was dissolved in 100 ml distilled water. The thiobarbituric acid which was in powder form was mashed finely to enable fast dilution. Dilution of this material was quite slow as it had to be placed at 50ºC for 1 hour or more until the 2-thiobarbituric acid fully dissolved.

20% Trichloroacetic acid
Trichloroacetic acid crystals (20.0 g) was dissolved in 100 ml distilled water.

24 mM $FeSO_4$
Light green colored $FeSO_4$ crystals (0.334 g) was dissolved in 50 ml of distilled water.

8. **Tyrosinase Inhibitory Assay**

Mushroom tyrosinase
The mushroom tyrosinase purchased was in the concentration 3933 units/mg. Hence, 1 mg of mushroom tyrosinase was diluted with 3.933 ml phosphate buffer (pH 6.8), to obtain mushroom tyrosinase stock with a concentration 1000 units/ml.

0.1 M Phosphate buffer (pH 6.8)
0.2M monobasic stock
13.9 g of sodium phosphate anhydrous monobasic of 15.6 sodium phosphate monobasic dehydrate is added with 500 ml distilled water
0.2M dibasic stock
53.65 g sodium phosphate dibasic heptahydrate is diluted with 1L distilled water or 28.4 g of anhydrous sodium phosphate dibasic with 1L distilled water.
0.1M phosphate buffer, pH 6.8
153.0 ml monobasic stock is added with 147.0 ml and 300 ml of distilled water is added. The pH is adjusted to 6.8 using a pH meter.

L-Tyrosine
L-tyrosine which acts as substrate is prepared at a concentration of 2.0mM. L-tyrosine powder (Sigma) (0.036 g) was diluted with 100 ml of 0.1M phosphate buffer solution (pH 6.8).

L-DOPA
L-DOPA which acted also as substrate was prepared at concentration of 12.0 mM. 0.2366 g of L-DOPA in powder form purchased from Sigma was diluted with 100 ml of 0.1 M phosphate buffer solution (pH 6.8).

Cytotoxicity Studies

Neutral Red Cytotoxicity Assay

Neutral Red Stock Solution
0.4g of Neutral Red (ICN, USA) was dissolved in 100 ml distilled water. The solution was kept at $4^{o}C$.

Neutral Red Medium
The Neutral Red stock solution (4mg/ml) was diluted (250µl) in treatment culture medium (20ml) to give a final concentration of 50µg/ml. The solution is then wrapped with aluminium foil and then centrifuged at 1000 rpm for 10 minutes before use to remove any fine, needle like precipitate of dye crystals.

Neutral Red Washing Solution
1.0g of calcium chloride ($CaCl_2$) Sigma was dissolved in 500µl formaldehyde (Sigma) and 99.5 ml distilled water. Solution was kept at $4^{o}C$.

Neutral Red Resorb Solution
1 ml of glacial acetic acid (BDH, AnalaR) was dissolved in 50 ml of absolute ethanol and 49 ml of distilled water.
Solution was kept at $4^{o}C$.

Basic (McCOY'S 5A, RPMI, MEM and M199) Medium
Medium was prepared by dissolved 11.9 g of (McCOY'S 5A, RPMI, MEM and M199) powder, 0.5206 g HEPES sodium salt (2mM/ L) and 2.0 g of sodium bicarbonate ($NaHCO_3$) in 1000 ml of distilled water. Then pH of the medium was calibrated to pH 7.4. The medium was then filter sterilized through a 0.22 µm filter membrane into sterile bottles and kept at $4^{o}C$.

10% supplemented medium (for cell maintenance)
10% of Supplemented Medium was prepared by using 90 ml of basic medium, supplemented with 10 ml inactivated Foetal Bovine Serum (FBS), 2 ml penicillin or streptomycin and 1 ml of Amphostat B (fungizone). The medium were filter sterilized using a 0.22 µm filter membrane. The colours of the medium were reddish orange and were kept at $4^{o}C$ for up to 2 weeks.

20% Supplemented Medium (for cell reviving)
20% of Supplemented Medium were prepared by using 50 ml of 10% Supplemented Medium added with 5 ml inactivated Foetal Bovine Serum (FBS). The medium were then filter sterilized using a 0.22 µm filter membrane. The colours of the medium were reddish orange and were kept at $4^{o}C$ for up to 2 weeks.

Phosphate Buffered Saline (PBS)
Preparation was done using 1.52 g of sodium phosphate anhydrous (Na_2HPO_4, BHD), 0.58 g of potassium dihydrogen orthophosphate (KH_2PO_4, BHD) and 8.5 g of sodium chloride (NaCl, Merck, Germany) that were dissolved in distilled water and the volume was made

up to 1 liter. The pH of the buffer was adjusted to 7.2. The buffer was then filtered using a 0.02 µm filter and stored at room temperature.

Phosphate Buffered Saline (PBS)

The Phosphate buffered saline (PBS) was prepared by dissolving 1.52g sodium phosphate anhydrous ($NaHPO_4$, Merck, Germany), 0.58g potassium dihydrogen orthophosphate (KH_2PO_4, Merck, Germany) and 8.5g sodium chloride (NaCl, BDH, AnalaR) in distilled water and the volume was made up to 1000ml. The pH of the buffer was adjusted to 7.2 by adding base or acid and monitored using pH meter. The buffer was then filtered by using a 0.22µm filter membrane and stored at room temperature.

Trypsin - EDTA

Trypsin - EDTA solution was prepared by dissolving 0.25g trypsin (Amresco, USA) and 0.03g EDTA (Sigma, USA) in 100ml of distilled water. The solution was sterilized by filtration using a 0.22µm filter membrane and stored at -20°C.

0.4% Tryphan Blue

0.4% Tryphan Blue solution was prepared by dissolving 0.2g tryphan blue in 50ml distilled water.

APPENDIX 3: Preparation of Apparatus

Washing of Apparatus

Glass and pipettes were soaked overnight in a diluted solution of 7X detergent (Flow Laboratories). They were rinsed thoroughly in tap water and followed by distilled water before drying in an oven (69ºC).

Sterilization

Pipettes were plugged with cotton wool and placed in metal canisters. They were sterilized in hot air oven at 180ºC for 2 hours. Bottles, pipettor tips and filter units were autoclaved at 15 psi at 121ºC for 20 minutes.